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PATENT
#96-0319-UNI
Case #F3141(V)

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on July 9, 1997

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Richard Anthony FENN; David NEEDHAM; Keith SMALLWOOD

Deposited: July 9, 1997

For: FROZEN FOOD PRODUCT

Edgewater, New Jersey 07020
July 9, 1997

SUBMISSION OF PRIORITY DOCUMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to rule 55(b) of the Rules of Practice in Patent Cases, Applicant(s) is submitting herewith certified copies of the European Application No. 96305498.6 filed July 26, 1996, upon which the claim for priority under 35 U.S.C. § 119 was made in the United States.

It is respectfully requested that the priority document be made part of the file history.

Respectfully submitted,

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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

96305498.6

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.: Application no.: Demande n°: 96305498.6

Anmeldetag: Date of filing: Date de dépôt: 26/07/96

Anmelder: Applicant(s): Demandeur(s):
UNILEVER PLC
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UNITED KINGDOM

Bezeichnung der Erfindung: Title of the invention: Titre de l'invention:
Frozen food with antifreeze peptides

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation: International Patent classification: Classification internationale des brevets:
A23G9/02

Am Anmeldetag benannte Vertragstaaten: Contracting states designated at date of filing: Etats contractants désignés lors du depôt: AT/BE/CH/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE

Bemerkungen: Remarks: Remarques: The original title of the application reads as follows : "Frozen food product".

Frozen Food product

Technical Field of the Invention

The invention relates to a process for the preparation of a food product containing AFP's and to food products containing AFP's.

Background to the Invention

Anti-freeze peptides (AFP's) have been suggested for improving the freezing tolerance of foodstuffs.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFP's can suitably be used in food-products such as ice-cream. Example 3B shows modified ice-crystal shapes if a water-ice mixture is frozen into a film in combination with 0.01 wt% of AFP.

WO 92/22581 discloses AFP's from plants which can be used for controlling ice crystal growth in ice-cream. This document also describes a process for extracting a polypeptide composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

WO 94/03617 discloses the production of AFP's from yeast and their possible use in ice-cream. WO 96/11586 describes fish AFP's produced by microbes.

Up till now, however the use of AFP's has not been applied to commercially available food products. One reason for this is that up till now it has proved

difficult to reproducibly ~~to~~ produce a frozen food product having the desired texture and eating characteristics.

The present invention aims at providing solutions to these problems. In particular the invention aims at providing frozen food products having a relatively hard and brittle texture, said texture being maintained upon prolonged storage at low temperatures.

Surprisingly it has been found that AFP's can conveniently be incorporated in frozen food products to result in the desired product properties as long as the processing conditions are varied such that the ice-crystal shape satisfies specific requirements.

Accordingly in a first aspect, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.

If food products are frozen, ice-crystals are formed throughout the product. If AFP's are included in food products to be frozen this generally leads to a favourable change in ice-recrystallisation properties. Aggregation of the ice-crystals of AFP containing products is believed to cause the brittleness of the product.

Many consumers are in favour of relatively hard and brittle frozen food products or ingredients such as ice-cream or water-ice. For example crispy water-ice can be used as an attractive ingredient in frozen confectionery products, also relatively hard ice-cream is liked by a large group of consumers.

Surprisingly we have found that AFP's offer the opportunity to formulate frozen food products which on the one hand are relatively hard and brittle and on the other hand retain improved ice-recrystallisation. Applicants have found that surprisingly this advantageous combination of properties can be achieved if the aspect ratio of the ice-crystals in the product is between 1.9 and 3.

The aspect ratio of ice-crystals is defined as the ratio of the length and the breadth of the ice-crystals. An aspect ratio of between 1.9 and 3 corresponds to elongated ice-crystals, which are not rounded in shape. The aspect ratio of crystals can be determined by any suitable method. A preferred method is illustrated in the examples. Preferably the ratio is between 2.0 and 2.9, most preferred between 2.1 and 2.8.

Preferably the frozen product of the invention are brittle. Preferably the minimum layer thickness at which fracture behaviour can be observed is less than 10 mm, more preferred from 1 to 5 mm. Fracture behaviour can either be measured by preparing layers of varying thickness and determining at which minimum thickness fracture behaviour occurs or calculated from the Young's Modulus as described in the examples.

During the formulation and subsequent freezing of food products several parameters can influence the aspect ratio of the ice-crystals to be formed. Examples of factors influencing the aspect ratio are given below. Applicants believe that it is well-within the ability of the skilled person to choose those conditions such that the aspect ratio of the ice-crystals falls within the desired range.

One factor influencing the aspect ratio of ice-crystals is the rate of freezing the product. Generally speaking an increase of the rate of freezing leads to a decrease in the aspect ratio for the ice-crystals.

Another factor influencing the aspect ratio of ice-crystals is the mobility of the product during freezing. For example if a liquid water-ice or ice-cream mix is to be frozen, quiescently freezing will lead to a fairly high aspect ratio for the ice-crystals, while stirring leads to a lower aspect ratio. High shear mixing will lead to even lower aspect ratios.

Another factor to influence the aspect ratio of the ice crystals is the presence and amounts of ingredients. For example the presence of ingredients which tend to form a network structure in the product (e.g. gums or fats) may lead to a lower aspect ratio than the in products without these ingredients. Also other ingredients may lead to lower aspect ratios, for example high solids levels e.g. high sugar levels may lead to low aspect ratios. Also high phase volumes for the ice may lead to higher aspect ratios.

Finally the nature and amount of the AFP's present may lead to a change in aspect ratios. Some AFP's seem to favour the formation of low aspect ratios, while other AFP's seem to induce higher aspect ratios. Variation in the amount of AFP's may lead to a change in aspect ratios.

According to a second embodiment, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the formulation, freezing and storage conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.

The process of the invention can be applied to any frozen food product containing AFP's. Examples of suitable products are sauces, meals etc. Preferred food products are frozen confectionery products such as ice-cream and water-ice.

Applicants have found that the AFP's for use in the process of the invention can come from a variety of sources such as plants, fishes, insects and microorganisms. Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFP's and the AFP's may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFP's as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (eg in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the

desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFP's are derived from fish. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our non-prepublished case PCT/EP96/02936.

For some natural sources the AFP's may consist of a mixture of two or more different AFP's.

Preferably those AFP's are chosen which have significant ice-recrystallisation inhibition properties. Preferably AFP's in accordance to the invention provide an ice particle size upon recrystallisation -as measured in accordance to the examples- of less than 20 μm, more preferred from 5 to 15 μm. It is believed that the small ice-crystal size combined with the specific aspect ratio is especially advantageous to obtain the desirable structural features.

A very advantageous embodiment of the invention relates to product formulations which are chosen such that in the preparation of the product quiescent freezing conditions can be used, while still obtaining the aspect ratio as defined above.

Examples of such food products are: frozen confectionery mixes such as ice-cream mixes and water-ice mixes which are intended to be stored at ambient or refrigerator temperature. Suitable product forms are for example: a powder mix which is packed for example in a bag or in sachets. Said mix being capable of forming the basis of the frozen food product e.g. after addition of water and optionally other ingredients and -optional- aeration.

Another example of a suitable mix could be a liquid mix (optionally aerated) which, if necessary after addition of further components and optional further aeration can be frozen.

The clear advantage of the above mentioned mixes is that the presence of the AFP ingredient enables the mixes to be frozen under quiescent conditions, for example in a shop or home freezer.

Very conveniently these mixes are packed in closed containers (e.g. cartons, bags, boxes, plastic containers etc). For single portions the pack size will generally be from 10 to 1000 g. For multiple portions pack sizes of up to 500 kg may be suitable. Generally the pack size will be from 10 g to 5000 g.

As indicated above the preferred products wherein the AFP's are used are frozen confectionery product such as ice-cream or water-ice. Preferably the level of AFP's is from 0.0001 to 0.5 wt% based on the final product. If dry-mixes or concentrates are used, the concentration may be higher in order to ensure that the level in the final frozen product is within the above ranges.

Surprisingly it has been found that compositions of the invention can contain very low amounts of AFP's while still being of good quality.

Up till now the general belief has been that fairly high levels of AFP's are required to obtain a reasonable improvement of recrystallisation properties. The reason for this is that it is commonly believed that the AFP's act on significant parts of the surface of the ice-crystals and therefore need to be present at fairly high levels e.g. 0.01 wt% or more to get a reasonable effect.

Surprisingly it has now also been found that for frozen products improved recrystallisation properties and increased temperature tolerance can already be obtained if low levels of AFP's are used.

Surprisingly it has been found that the level of AFP's can be as low as 0.1 to 50 ppm while still providing adequate recrystallisation properties and temperature tolerance in frozen confectionery products. Although applicants do by no means wish to be bound by any theory, the reason for this may be that the interaction between the solids of the frozen confectionery and the AFP's provides an excellent mechanism for inhibiting crystal growth. Most conveniently the level of AFP is from 1 to 40 ppm, especially preferred from 2 to 10 ppm.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees.

Preferably ~~a~~ the level of solids in the frozen confection (e.g. sugar, fat, flavouring etc) is more than 30 wt%, more preferred from 40 to 70wt%.

In a very preferred embodiment of the invention the hard and crispy frozen confectionery formulations are used to create texture contrast in ice confections. Preferably such ice-confections contain as discrete elements in their structure the AFP containing composition in accordance to the invention. For example a relatively soft ice-cream core can be coated with a thin layer of the composition of the invention therewith providing a relatively hard and crispy layer surrounding the ice-cream core. Another embodiment could be the incorporation

of the formulation of the invention as inclusions in ice-confections. A third embodiment would be the alternating of layers of ice-cream with the formulation of the invention to create thin crispy layers alternating with the ice-cream layers.

Example I

A pre-mix for preparing ice-cream was made by mixing:

Ingredient	**% by weight**
Skimmed milk powder	10.00
sucrose	13.00
maltodextrine (MD40)	4.00
Locust bean gum	0.14
butteroil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (Type III HPLC-12)	0.01 or none(control)
water	balance

This mix can conveniently be stored at ambient temperature e.g. in a plastic container.

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65 °C followed by ageing over night at 5°C. The mix was frozen using a freezer (MF50 SSHE Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature was -4.5°C, the overrun was 110%. The product is then frozen at -35°C and stored at -80°C.

After two months storage the composition according to the invention had a markedly better texture than the control sample.

Example II

An ice-cream was prepared of the following formulation:

Ingredient	**% by weight**
Skimmed milk powder	10.00
sucrose	13.00
maltodextrine (MD40)	4.00
Locust bean gum	0.14
butteroil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (Type III HPLC-12)	0.01 or none(control)
water	balance

The method of preparation was as in example I.

Samples of both products were equilibrated at -18°C in a Prolan environmental cabinet for approximately 12 hours. Microscopic slides were prepared by smearing a thin layer of ice-cream from the centre of thin glass plates.

Each slide was transferred to a temperature controlled microscopic stage (at -18°C) where images of ice-crystals (about 400 individual ice-crystals) were collected and relayed through a video camera to an image storage and analysis system.

The stored ice crystal images were highlighted manually by drawing around its perimeter which then highlights the whole crystal. Images of the highlighted crystals were then measured using the image analysis software which counts the number of pixels required to complete the longest straight line (length), shortest straight line (breadth), the aspect ratio (length/breadth).

The average aspect ratio for the crystals was calculated.

For the control sample the aspect ratio was 1.45

For the sample containing AFP the aspect ratio was 2.24.

Example III

The brittleness of the ice-cream of example IV was determined by calculations on the fracture behaviour of the ice-cream. Using a 3-Point bend test the Young's Modulus was measured.

The Young's modulus was measured by preparing strips of ice-cream, equilibrating them for 18 hours in a freezer cabinet and transferring to a temperature cabinet. The strips were placed on a 3-point bend rig as described in Handbook of Plastics Test Methods (2nd Edition), ed R.P. Brown, George Godwin Ltd, 1981. Sample testing was carried out immediately at a deformation speed of 50 mm/min. From the force-deformation curve, the initial slope was measured and used to calculate the Young's modulus according to the following equation:

$$\text{Young Modulus (Pa)} = \frac{\text{Slope}. L^3}{4. B. W^3}$$

- where L = beam span (110mm), B = sample width, W = sample height. Usually eight samples were tested to give a mean Young's Modulus value.
Using the calculations described by Williams & Cawood in Polymer Testing 9 15-26 (1990) the fracture toughness can be calculated.

The results were as follows: For the control sample a thickness of 966 m was calculated as being necessary to obtain a brittle layer. For the AFP containing sample brittleness (fracture behaviour) was already found at a thickness of 3 mm.

This clearly shows the improved brittleness of products of the invention.

Claims

1. A process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.

2. A process according to claim 1 wherein the conditions for influencing the aspect ratio are selected from the group of: rate of freezing, mobility of product during freezing, storage temperature and time, formulation of the product and nature and amount of AFP's and combinations thereof.

3. A process according to claim 1 or 2, wherein the frozen food product is a frozen confectionery product.

4. A frozen confectionery product comprising from 0.0001 to 0.5 wt% of AFP's, said product having an ice-crystal aspect ratio of from 1.9 to 3.0.

5. Frozen confectionery product having a texture contrast, said product comprising disrete elements of a confectionery product of claim 4.

6. Frozen confectionery product according to claim 5, comprising thin ice-cream layers alternating with thin water-ice layers, wherein the water-ice layers comprise from 0.0001 to 0.5 wt% of AFP's and have an ice-crystal aspect ratio of from 1.9 to 3.0.

7. Ice-cream mix suitable for use in the preparation of a frozen confectionery product of claim 4.

8. Ice-cream mix according to claim 7 wherein the preparation involves (optional) aeration and quiescent freezing.

Abstract

A process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.9 to 3.